

## A New Host Record for the Afrotropical Parasitic Wasp Genus *Bathyaulax* Szépligeti (Hymenoptera: Braconidae: Braconinae) Confirmed using DNA Sequence Data

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*Abstract.*—An apparently undescribed species of the braconid wasp genus *Bathyaulax* Szépligeti from Kibale National Park, Uganda, is recorded as a parasitoid of the callichromine cerambycid beetle, *Chromalizus fragrans* (Dalman) subspecies *cranchi* (White), a borer of ‘forest mahogany’ tree, *Trichilia dregeana* Sond. (Meliaceae). The association was confirmed using DNA sequencing of a parasitoid pupa recovered from the pupal chamber of the beetle. A gabuniine cryptine ichneumonid wasp, *Gabunia* aff. *togoensis* Krieger, was also confirmed as a parasitoid of the same beetle. These rearings are discussed in the light of previous host records.

*Key words.*—Parasitoid, DNA, *Gabunia*, Ichneumonidae, *Chromalizus*, Cerambycidae, *Trichilia*, Meliaceae, mahogany.

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Of the approximately 40 genera of Braconinae that are believed to be endemic to the Afrotropical region (Quicke 1987), host records are available for fewer than five, and some of those records that have been published may well be erroneous. Common problems with records of host-parasitoid associations include misidentifications of the parasitoid, or of the host or misidentification of both, or wrong associations, especially when substrate rearings are involved (Noyes 1994; Shaw 1994, 2003; Quicke 2003). Whilst the majority of braconines are parasitoids of concealed hosts, usually larvae of wood-boring beetles for larger species and groups such as stem-borers, leaf-rollers and leaf-miners for smaller ones, the lack of reliable host data for the great majority of species is hampering our understanding of the factors that permit coexistence of so many species of parasitoids with essentially similar life histories.

DNA sequencing provides a powerful tool to confirm host parasitoid associations where cryptic hosts are involved because it enables identification of otherwise, at least currently, unidentifiable host and parasitoid immature stages (Quicke et al. 2003; Laurenne & Quicke 2004). This can be likened to a bar-coding approach (Hebert et al. 2003a,b), and in principle food-webs can be constructed and analysed largely based on taxa whose actual identities are only known approximately—appropriate DNA sequences will usually allow placement to genus or species group. For tropical studies, this actually may not be very different from current morpho-taxonomic studies, as many of the taxa are likely unidentifiable to anything more precise than morpho-species, and even generic placement can sometimes be problematic for many less well-known insect groups.

Here we use DNA sequencing to con-

firm a host-parasitoid relationship for *Bathyaulax* Szépligeti, a poorly known Afrotropical genus of large parasitic braconid wasps, and additionally reveal that a second parasitoid, a member of the cryptine ichneumonid genus *Gabunia* Kriechbaumer, is also involved in the system. The observations also provide a new host tree record for the longhorn beetle involved. Because the tree, 'forest mahogany', is noted for its high quality wood, this may be of some economic significance.

#### MOLECULAR MATERIALS AND METHODS

We followed the procedures in Laurenne et al. (2000) to obtain 28S D2–D3 rDNA sequence data from the adult *Bathyaulax* and two of the immature parasitoids found in the host's pupation chambers. The sequences for the adult *Bathyaulax* sp. and the presumed conspecific pupa have GenBank/EMBL accession numbers AY604326 and AY604327 respectively. The sequence of the ichneumonid larva was virtually identical to ones obtained for two Afrotropical *Gabunia* species that will be reported elsewhere as part of a molecular phylogeny of the Cryptinae (GenBank/EMBL accession numbers AY527195 and AY527196; Laurenne, Broad & Quicke, submitted). Additional braconine 28S rDNA sequences used (EMBL accession numbers given in parentheses) for comparison are: *Bathyaulax* sp., Kenya (AJ231501), *Bathyaulax cyanogaster* Szépligeti, Nigeria (AY527194), *Monilobracon* sp., Sierra Leone, Lumley (AJ296046), *Iphiaulax* sp., Africa (AJ296052), *Merinotus* sp., Uganda, Kibale (AY296649), *Plaxopsis* sp., Africa (AJ231533), *Latana keijua* Laurenne & Quicke, Uganda, Kibale (AY296638), *Odontoscopus* sp., Kenya (AJ231503), *Ipobracon* sp. 1, Uganda, Kibale (AY296642), *Ipobracon* sp. 2, Uganda, Kibale (AY296641), *Archibracon deliberator* Szépligeti, Benin (AY529702), *Sororarchibracon* sp. Africa (AY296645), *Sylvibracon* sp., Africa (AY296643).

#### FIELD SITE

Observations were made near Makerere University Biological Field Station, Kanyawara, Kibale National Park, West Uganda, in an area of montane rain forest that was lightly logged in 1960 (Area 14 in Struhsaker 1997).

#### OBSERVATIONS, RESULTS AND DISCUSSION

A large black and red female parasitic wasp was noticed 'drilling' into a dead trunk (6cm diameter) of a young 'forest mahogany' tree, *Trichilia dregeana* Sond. (Meliaceae), that appeared to have been felled during path clearing earlier in the year. The wasp, identified as a species of *Bathyaulax*, was collected and the point of ovipositor drilling noted and marked. Two days later a length of the dead tree was cut and taken to a more convenient site for dissection. This approximately 2m long stretch of the wood was found to be heavily bored by larvae of the longhorn beetle *Chromalizus fragrans* (Dalman) subspecies *cranchi* (White) (Cerambycidae: Cerambycinae: Callichromini); sometimes this subspecies is treated as a full species. The beetle is recorded by Duffy (1957) as being widely distributed in central and western equatorial Africa, viz. Angola, Democratic Republic of Congo (as Belgian Congo), Cameroons, Ghana (as Gold Coast) and Rio Muni. Its host plants are reported to include *Coffea canephora* Pierre ex Froehner (as *robusta* Linden) and *Teclea viridis* Verdoorn, but it has not previously been reported from any *Trichilia* species (Duffy 1957). Most Callichromini attack their host trees while they are still alive, supporting our assumption that the tree involved had been alive until fairly recently before we discovered it.

In total the borings included three with beetle pupae, two of which were extracted alive and reared for identification. In addition to these beetle pupae, three of the beetle pupation chambers contained par-

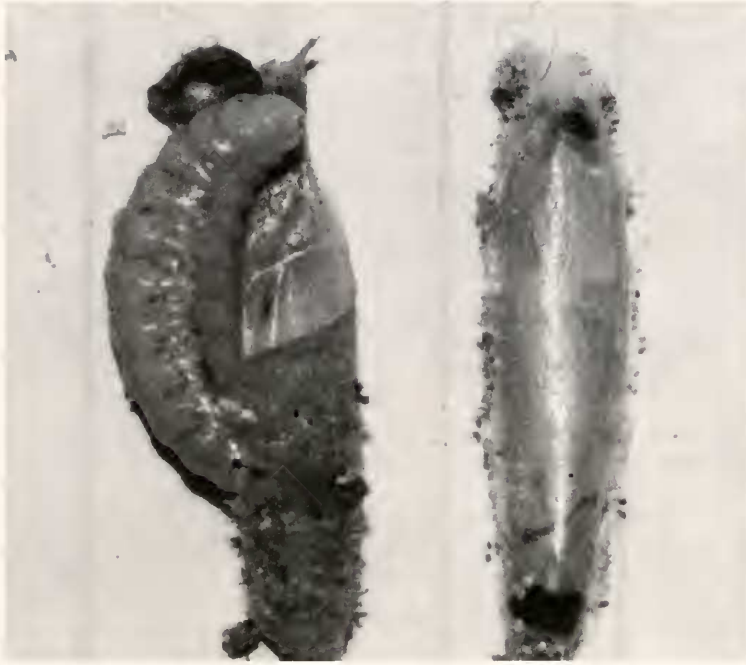


Fig. 1. Photographs of parasitoid cocoons found in *Chromalizus fragrans* pupation chambers: *Gabunia* sp. (Ichneumonidae: Cryptinae) on left, removed from cocoon; *Bathyaulax* sp. (Braconidae: Braconinae) pupa in cocoon on right.

asitoids. One contained a large parasitoid pupa at an advanced stage of development judging from its pigmentation within its reddish-brown silk cocoon, the second contained a large whitish larva within a reddish-brown cocoon within which there was also a reddish meconial pellet (Fig. 1 left), and the third contained a more slender, pale yellow parasitoid pupa within a pale yellow brown cocoon (Fig. 1 right). The beetle pupae and the parasitoid cocoons were all found in cells behind a hard, concave, white, rather crystalline deposit (probably a meconial material from the beetle larva) that sealed the chamber off from the rest of the boring.

The well-developed parasitoid from the red-brown cocoon emerged (though rather deformed) after approximately 2 weeks and was identified as *Gabunia* aff. *togoensis* Krieger (Ichneumonidae: Cryptinae), a species that is quite common in Kibale and which probably relies largely on vibra-

tional sounding to locate hosts (Quicke et al. 2003). The host remains associated with it show that it had attacked its host in the larval or pre-pupal stage.

DNA sequencing of the cocooned parasitoid larva in the reddish-brown cocoon and of the yellow parasitoid pupa revealed these to be a *Gabunia* species and a *Bathyaulax* species respectively. Some of the molecular data supporting the latter identification are shown in Fig. 2; the other sequence was identical to that obtained from the *Gabunia* aff. *togoensis* specimen whose behaviour was reported by Quicke et al. (2003). To date three *Bathyaulax* species, collectively from different parts of the Afrotropical Region, have been sequenced, and all share a very similar insert region (Fig. 2). Apart from that, the three sequences, which include the very morphologically aberrant *B. cyanogaster* Szépligeti, are virtually identical, though interspecific differences are also apparent.

Wasp	Gene fragment
<i>Bathyaulax</i> sp., Kibale	TATGGGTTACT---ACTACAG- <b>TTAC</b> --- <b>T</b> ---GTA-GT---GT-----GTATTGCC
<i>Bathyaulax</i> sp., Kenya	TATGGGTTACT--CTG <b>CTACAA</b> - <b>TTAC</b> --- <b>T</b> ---GTA-GTAC-GT-----GTATTGCC
<i>Bathyaulax cyanogaster</i>	TATGGGTTACT--CTACT <b>ACAA</b> - <b>TTAC</b> --- <b>T</b> ---GTA-GTACTGT-----GTATTGCC
<i>Monilobracon</i> sp.	TATGGGTTACT-----TTTACAC--CTTGTGTGTATT-----AT---GTATTGCC
<i>Latana keijua</i>	TATGGGTTACTATATGCATATATGCAC---TTGT--GTATGTA-TGT---TTAGTATTGCT
<i>Odontoscopus</i> sp.	TATGGGTTACTATTCTTTAATCGG-TC---TT-----A-----GTATTGCC
<i>Iphiaulax</i> sp.	TATGGGTTACT-----AC---TT---GTA-----GTATTGCC
<i>Merinotus</i> sp.	TATGGGTTACT-----AC---TT---GTA-----GTATTGCC
<i>Plaxopsis</i>	TATGGGTTACT-----AC---TT---GTA-----GTATTGCC
<i>Ipobracon</i> sp.1	TATGGGTTACT-----AC---TTA-T-GTA-----GTATTGCC
<i>Ipobracon</i> sp.2	TATGGGTTACT-----AC---TT---GTA-----GTATTGCC
<i>Archibracon deliberator</i>	TATGGGTTACT-----AC---TT---GTA-----GTATTGCC
<i>Sororarchibracon</i> sp.	TATGGGTTACT-----AC---TT---GTA-----GTATTGCC
<i>Sylvibracon</i> sp.	TATGGGTTACT-----AC---TT---GTA-----GTATTGCC

Fig. 2. By eye arrangement of part\* of 28S D2 rDNA sequence from the Kibale specimens (adult and pupa) of *Bathyaulax*, plus two other congeners and various taxa of large braconines representing genera found at Kibale. Features unique to *Bathyaulax* are highlighted in bold face.

\*, corresponding to part starting at position 23 in the alignment shown in Fig. 1 of Belshaw et al. (1998) and running into first box.

Previously *Bathyaulax* species have been reared on only three occasions (Quicke 1989). On two of these, they were reared from *Acacia* pods in Namibia (recorded as South West Africa) and in one of these two cases the host was reported as being a larval cerambycid. In the third known rearing, made in Kenya, the host was identified as the cerambycid beetle *Enaretta castelnaudi* Thomson (Cerambycidae: Lamiinae: Tetraopini) also on *Acacia*. *Enaretta castelnaudi* is widespread throughout the Afrotropical region and its larvae feed on the seeds of several *Acacia* species including *A. albida* Rojas, *A. hebeclada* DC and *A. stolonifera* Burch. (Duffy 1957). Thus *Bathyaulax* species appeared to be associated with hosts in *Acacia* seed pods, and the large arched terminal tarsal articles and slender claws of *Bathyaulax* species (Quicke 1981) could reasonably be interpreted as being adaptations to gripping these hard and smooth host substrates. However, no data are available for *Bathyaulax* species dwelling in moist tropical

forests where *Acacia* is effectively absent, though other pod-bearing trees are of course present. The present observations show that *Bathyaulax* species are not restricted to attacking hosts in pods though an exclusive association with Cerambycidae is still possible. It seems unlikely that the arched telotarsi would be of any special use in the species involved here as the hosts were inside a normal piece of wood, albeit one of a relatively hard type.

No meaningful key to species of *Bathyaulax* exists and therefore it is not possible to identify the species involved here. Although Fahringer (1928) provided keys, based very largely on the original literature sources rather than examination of specimens, he split members of the genus mostly between *Bathyaulax*, *Megagonia* Szépligeti and *Goniobracon* Szépligeti, and included within each of these members of other braconine genera with large anterolateral areas on the 3rd metasomal tergite such as *Zaglyptogastra* Ashmead species, though these appear to be but distantly re-



lated to *Bathyaulax*. The list of species in Shenefelt's (1978) catalogue is therefore effectively meaningless. The type specimens of virtually all described species of tropical Braconinae have been examined by the senior author, along with all the original descriptions, and as a result approximately 35 species are now placed in *Bathyaulax* (see Quicke 1981, 1983, 1985a,b, 1991a, Quicke & van Achterberg 1990, Quicke & Koch 1990). Based on these observations, the species of *Bathyaulax* dealt with here appears to be new to science and will be dealt with in a forthcoming revision of the genus (Kaartinen & Quicke in prep.). Indeed, although revisions of genera of large and colourful ichneumonoids from the tropics often leads to no major increase in the total number of known species (e.g. El-Heneidy and Quicke 1991; Quicke 1991b; Chishti and Quicke 1994, 1996), much of the material collected by the authors in Kibale in 2002 and 2003 seems to be new, and it is possible that this represents a relatively distinct and very little studied ichneumonoid fauna (as also indicated by its cryptine ichneumonid fauna; Gavin Broad pers. comm.).

*Trichilia* P. Browne, the host tree genus including the widespread Afrotropical species involved here, is known for its very good, rather dense and close-grained wood that is widely used for making furniture and utensils, but is reported to be heavily attacked by wood-borers (Noad and Birnie 1989; Beentje 1994). Further, several species in this Afrotropical and Neotropical genus have been subject to intense investigation as a possible source of biologically active compounds, both as potential medical and potential crop protection agents. Further investigation of its borer and associated parasitoid systems will therefore be particularly interesting.

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